

IN THE CLAIMS

Please cancel claims 1, 5-12 and 23-26. Please amend claims 2-4, 13-22 and 27-31. Claim 2 incorporates subject matter of claim 12. Claim 3 incorporates subject matter of claim 9.

1. (cancelled)

2. (presently amended) A method for site-specific incorporation of acyclonucleotides into DNA, comprising:
reacting an archaeon Family B DNA polymerase with a primed DNA template and nucleotide solution containing at least one acyclonucleotide to produce fragments of DNA with the acyclonucleotide covalently attached to the 3' terminal residue;

wherein the DNA polymerase is encoded by an isolated DNA fragment that hybridizes in a Southern blot to an isolated DNA fragment selected from the group consisting of a DNA fragment having nucleotides 1-1274 of SEQ ID NO:4, a DNA fragment having nucleotides 291-1772 of SEQ ID NO:4, a DNA fragment having nucleotides 3387-3533 of SEQ ID NO:4, a DNA fragment having nucleotides 4704-5396 of SEQ ID NO:4, and a DNA fragment having nucleotides 4718-5437 of SEQ ID NO:4, wherein hybridization is conducted under the following conditions: a) hybridization: 0.75 M NaCl, 0.15 M Tris, 10 mM EDTA, 0.1% sodium pyrophosphate, 0.1% sodium lauryl sulfate, 0.03% BSA, 0.03% Ficoll 400, 0.03% PVP and 100 µg/ml boiled calf thymus DNA at 50°C for about 12 hours and; b) wash: 3X30 minutes with 0.1X SET, 0.1% SDS, 0.1% sodium pyrophosphate and 0.1 M phosphate buffer at 45°C.

3. (presently amended) A method for site-specific incorporation of ~~derivatized~~ acyclonucleotides into DNA, comprising:
reacting an archaeon Family B DNA polymerase with a primed DNA template and a nucleotide solution containing at least one ~~derivatized~~ acyclonucleotide to produce fragments of DNA with the ~~derivatized~~ acyclonucleotide covalently attached to the 3' terminal residue;
wherein the DNA polymerase has at least 30% primary amino acid sequence identity with Vent DNA polymerase.

4. (presently amended) The method of claims ~~1~~ 2 or 3 wherein the ~~derivative~~ acyclonucleotide comprises a detection reagent.

5-12 (cancelled)

13. (presently amended) The method of claims ~~1-3~~ 2, wherein the DNA polymerase is selected from the group consisting of Vent, Deep Vent, Pfu and 9°N DNA polymerases.

14. (presently amended) The method of claims ~~1-3~~ 2 or 3, wherein the DNA polymerase has been mutated by substitution of an amino acid residue at a site corresponding to A488, L492, A493 and Y499 in Vent polymerase.

15. (presently amended) The method of claims ~~1-3~~ 2 or 3, wherein the DNA polymerase has been mutated by substitution of an amino acid residue corresponding to A488 in Vent polymerase with L, I, V, F, S or C.

16. (presently amended) The method of claims ~~1-3~~ 2 or 3, wherein the DNA polymerase has been mutated by substitution of an amino acid residue corresponding to A488 in Vent DNA polymerase with L.

17. (presently amended) The method of claims ~~1-3~~ 2 or 3, wherein the DNA polymerase has been mutated by substitution of an amino acid residue corresponding to Y499 in Vent DNA polymerase with L.

18. (presently amended) The method of claims ~~1-3~~ 2 or 3, wherein the DNA polymerase is a mutant selected from the group consisting of Vent (A488L), Vent' (Y499L) and 9°N (A485L) DNA polymerases.

19. (presently amended) The method of claims 2 or 3, wherein the acyclonucleotide is incorporated to an extent greater than that of a corresponding dideoxynucleotide.

20. (presently amended) The method of claims 2 or 3 wherein the acyclonucleotide is incorporated to an extent of at least, approximately, two-fold greater than incorporation of a corresponding dideoxynucleotide.

21. (presently amended) The method of claims 2 or 3 wherein the acyclonucleotide is incorporated to an extent at least, approximately, five-fold greater than incorporation of the corresponding dideoxynucleotide.

22. (presently amended) The method of claims 2 or 3 wherein the acyclonucleotide is incorporated to an extent at least, approximately, nine-fold greater than incorporation of the corresponding dideoxynucleotide.

23-26 (cancelled)

27. (presently amended) The method of claims ~~1-3~~ 2 wherein the DNA polymerase is ~~additionally~~ thermostable.

28. (presently amended) The method of claims ~~1-3~~ 2 wherein the DNA polymerase has no detectable exonuclease activity.

29. (presently amended) The method of claims ~~1-3~~ 3 wherein the DNA polymerase has an exonuclease activity of less than about 5% of Vent polymerase ~~the exonuclease activity of the unmodified enzyme.~~

30. (presently amended) The method of claims ~~1-3~~ 3 wherein the DNA polymerase has an exonuclease activity of less than about 25% of Vent polymerase ~~the exonuclease activity of the unmodified enzyme.~~

31. (presently amended) The method of claims ~~1-3~~ 2 further comprising the step of employing the resulting sequence-specific termination product or products in DNA sequence determination.